

10/540092

Amendments to the Specification:

JC17 Rec'd PCT/P10 20 JUN 2005

Please replace the paragraph on page 9 (lines 18-20) as follows:

Fig. 2: Conserved core motifs between NRPSs. Conserved amino acid sequences in SacA (Residues 484-953 of SEQ ID NO: 2), SacB (Residues 525-999 of SEQ ID NO: 3) and SacC (Residues 516-992 of SEQ ID NO: 4) proteins and their comparison with its homologous sequences from *Myxococcus xanthus* DM50415 (Core sequences disclosed as SEQ ID NOS: 26-30; SafB1, SafB2, SafA1 and SafA2 disclosed as SEQ ID NOS: 31-34, respectively, in order of appearance).

Please replace the paragraph on pages 14 (lines 8-28) through 15 (lines 1-5) as follows:

The first surprising feature of the safracin NRPS proteins is that from the known active sites and core regions of peptide synthetases (Konz and Marahiel, Chem. and Biol. 1999, 6, R39-R48), the first core is poorly conserved in all three peptide synthetases, SacA, SacB and SacC (Fig. 2). The other five core regions are well conserved in the three safracin NRPSs genes. The biological significance of the first core (LKAGA; SEQ ID NO: 16) is unknown, but the SGT(ST)TGxPKG (SEQ ID NO: 17) (Gocht and Marahiel, J. BacteHol. 1994, 176, 2654-266; Konz and Marahiel, Chem. and Biol. 1999, 6, R39-R48), the TGD(Gocht and Marahiel, J. Bactetiol. 1994, 176, 2654-2662; Konz and Marahiel, 1999) and the KIRGxRIEL (SEQ ID NO: 18) (Pavela-Vrancic et al. J. Biol. Chem 19942 269, 14962-14966; Konz; and Marahiel, Chem. and Biol. 1999, 6, R39-R48) core sequences could be assigned to ATP binding and hydrolysis. The serine residue of the core sequence LGGxS (SEQ ID NO: 19) could be shown to be the site of thioester formation (D'Souza et al., J. Bacteriol. 1993, 175, 3502 3510; Vollenbroich et al., FEBS Lett. 1993, 325(3), 220-4; Konz and Marahiel, Chem. and Biol. 1999, 6, R39-R48) and 4'-phosphopantetheine binding (Stein et al. FEBS Lett. 1994, 340, 39-44; Konz and Marahiel, Chem. and Biol. 1999, 6, R39-R48).

These findings, together with the fact that safracin seems to be synthesized from amino acids, supports the hypothesis that non-ribosomal peptide bond formation via the thiotemplate mechanism is involved in the biosynthetic pathway of safracin and that *sacA*, *sacB* and *sacC* encode the corresponding peptide synthetases. According to this mechanism, amino acids are activated as aminoacyl adenylates by ATP hydrolysis and subsequently covalently bound to the enzyme via carboxyl-thioester linkages. Then, in further steps, transpeptidation and peptide bond formation occurs.

Please replace Table III on page 25 as follows:

Table III. PCR primers designed for this study.

(SEQ ID NOS: 20 and 21, respectively, in order of appearance)

Primer designation and orientation	Sequence	Length
PS34-YG (forward)	5'- TAYGGNCCNACNGA -3'	14-mer
PS6-FF (reverse)	5'-TSNCCNCCNADNTCRAARAA-3'	20-mer

Please replace the paragraph on page 49 (lines 1-20) as follows:

To overproduce P14, *sacEFGH* genes were cloned (pB7983) (Fig. 4). To overproduce P2 in a heterologous system, *sacD* to *sacH* genes were cloned (pB51183)(Fig. 4). For this purpose we PCR amplified fragments harboring the genes of interest using oligonucleotides that contain a *Xba*I restriction site at the 5' end. Oligonucleotides PFSC79 (5'

CGTCTAGACACCGGCTTCATGG-3' SEQ ID NO: 22 and PFSC83 (5p GGTCTAGATAACAGCCAACAAACATA-3 SEQ ID NO: 23) were used to amplify *sacE* to *sacH* genes; and oligonucleotides 5HPTI-XB (5'-CATCTAGACCGGACTGATATTG-3' SEQ ID NO: 24) and PFSC83 (5'- GGTCTAGATAACAGCCAACAAACATA-3' SEQ ID NO: 25) were used to amplify *sacD* to *sacH* genes. The PCR fragments digested with *Xba*I

were cloned into the *Xba*I restriction site of the pBBR1-MCS2 plasmid (Kovach et al, Gene 19942 166p 175-176). The two plasmids, p137983 and pB5H83, were introduce separately into three heterologous bacteria *P. fluorescens* (CECT 378), *P. putida* (ATCC12633) and *P. stutzeri* (ATCC 17588) by conjugation (see table II). When culture broth of the fermentation of the transconjugant strains was checked by HPLC analysis, big amounts of P14 compound was visualized in the three strains containing pB7983 plasmid, whereas big amounts of P2 and some P14 product were observed when pB5H83 plasmid was expressed in the heterologous bacteria.